

Chromosomes of Some Opisthobranchiate Mollusks from Eniwetok Atoll, Western Pacific¹

J. B. BURCH² and R. NATARAJAN³

ABSTRACT: Chromosome numbers are reported for nine species of opisthobranchiate mollusks from Eniwetok Atoll, Marshall Islands, western Pacific. In the Nudibranchia, both *Dendrodoris nigra* (Dendrodorididae) and *Herviella mietta* (Favorinidae) were found to have 13 bivalents during male meiosis. In the Anaspidea, *Dolabrifera dolabrifera* and *Stylocheilus longicauda* (Aplysiidae) both had 17 bivalents during male meiosis. In the Cephalaspidea, *Haminoea linda* and *H. musetta* (Atyidae) each had 17 pairs of chromosomes during male meiosis and *Lathophthalmus smaragdinus* and *Smaragdinella calyculata* (Smaragdinellidae) had 18 pairs. In the Soleolifera, *Onchidella evelinae* had 18 bivalents during male meiosis.

The extreme conservativeness of chromosome numbers in opisthobranchiate mollusks is demonstrated by that fact that all 18 nudibranchs from 10 families studied so far have the single haploid chromosome number 13, and that 18 of the 21 species of the orders Entomotaeniata, Anaspidea, Cephalaspidea, and Sacoglossa have 17 pairs of chromosomes. The haploid number 18 is here reported for the first time for nonsoleoliferan opisthobranchiate mollusks. The more advanced, mostly fresh-water, order Basommatophora, in which the haploid number 18 is the basic number, may well have been derived from a taxon within or related to this cephalaspid superfamily (Philinacea).

IN RECENT YEARS, detailed investigations have been made on the chromosomes of many basommatophoran and stylommatophoran snails (Burch, 1965), but relatively few species of the opisthobranchiate⁴ orders have been studied,

mainly because of the difficulties they present in collection and identification. Relying on the studies of various authors during the early part of the present century, Makino (1951) listed the chromosome numbers of 16 opisthobranchiate species, but recent investigations by Inaba and our present studies indicate that the earlier records are not dependable and so are obsolete. Previous reliable reports on the chromosomes of opisthobranchiate gastropods are those of Inaba and Hirota (1954, 1958), Inaba (1959a, 1959b, 1961), Natarajan (1959, 1960), Mancino and Sordi (1964a and b), and Burch (1965). These authors give the chromosome numbers of 36 species belonging to 21 families and 7 orders (Tables 1 and 2). This is a very small number when compared with the great multitude of species currently recognized in the opisthobranchiate orders. The present paper presents the chromosome numbers of 9 opisthobranchiate

¹ The field work for this investigation was supported by the Division of Biology and Medicine, U. S. Atomic Energy Commission. The research was supported (in part) by grants from the National Science Foundation, Washington, D. C. (GB 787) and the National Institute of Allergy and Infectious Diseases, U. S. Public Health Service (5 T1 AI 41-08). Manuscript received March 14, 1966.

² Museum and Department of Zoology, University of Michigan, Ann Arbor. Supported by a Public Health Service research career program award (No. 5-K3-AI-19, 451-03).

³ Museum of Zoology, University of Michigan, Ann Arbor.

⁴ Boettger (1955) considers the orders Opisthobranchiata and Pulmonata (together equivalent to the subclass Euthyneura) to be unnatural ones, and instead divides the Euthyneura into seven orders. While we do not disagree with this, it is still convenient to speak of his five lower (mainly marine) euthyneuran orders (Nudibranchia, Soleolifera, Cephalaspidea, Sacoglossa, and Anaspidea) as "opistho-

branches" as distinguished from the more advanced Basommatophora (mainly freshwater) and Stylommatophora (land inhabitants).

TABLE 1
CHROMOSOME NUMBERS PREVIOUSLY REPORTED IN THE OPISTHBRANCHIATE ORDERS
NOTASPIDEA AND NUDIBRANCHIA

SPECIES	CHROMOSOME NUMBER		LOCALITY	REFERENCE
	2n	n		
NOTASPIDEA				
Pleurobranchidae				
<i>Pleurobranchaea novaezealandiae</i>	24	12	Japan	Inaba, 1959a
NUDIBRANCHIA				
Dorididae				
<i>Glossodoris festiva</i>		13	Japan	Inaba and Hirota, 1958
<i>Glossodoris pallescens</i>		13	Japan	Inaba and Hirota, 1954; 1958
<i>Rostanga arbutus</i>	26	13	Japan	Inaba, 1959a
<i>Discodoris pardalis</i>		13	Japan	Inaba, 1959a
<i>Doris verrucosa</i>	26	13	Italy	Mancino and Sordi, 1964a
Dendrodorididae				
<i>Dendrodoris miniata</i>	26	13	Japan	Inaba and Hirota, 1958
<i>Dendrodoris nigra</i>	26	13	Japan	Inaba and Hirota, 1958; Inaba, 1959b
Triophidae				
<i>Kaloplocamus ramosus</i>		13	Japan	Inaba and Hirota, 1958
<i>Plocamopherus tilesii</i>	26	13	Japan	Inaba and Hirota, 1958
Goniodorididae				
<i>Okenia barnardi</i>		13	Japan	Inaba and Hirota, 1958
Fimbriidae				
<i>Melibe papillosa</i>	26	13	Japan	Inaba, 1959a
Dotonidae				
<i>Doto bella</i>		13	Japan	Inaba, 1961
Arminidae				
<i>Dermatobranchus striatus</i>	26	13	Japan	Inaba and Hirota, 1958
Cuthonidae				
<i>Catriona pupillae</i>		13	Japan	Inaba, 1961
Facelinidae				
<i>Facelina ceylonica</i>		13	Japan	Inaba and Hirota, 1958
<i>Facelina japonica</i>		13	Japan	Inaba and Hirota, 1958

species belonging to 6 families and 4 orders (Table 3), which were collected in shallow waters around three islands of Eniwetok Atoll in the western Pacific by the senior author and Dr. William H. Heard during early 1960.

MATERIAL AND METHODS

The species studied in this investigation and the localities where they were collected are listed below. Duplicate specimens have been deposited in the collections of the Museum of Zoology at the University of Michigan, the University of São Paulo, and the University of Hawaii.

NUDIBRANCHIA

1. *Dendrodoris nigra* (Stimpson) (Fig. 1). North end of Japtan Island, under loose pieces of dead coral. April 1, 1960.

2. *Herviella mietta* Marcus and Burch (Fig. 2). North end of Eniwetok Island on the lagoon side, in about 10 cm of water at low tide, under submerged pieces of dead coral. April 2, 1960.

ANASPIDEA

3. *Dolabrifera dolabrifera* (Rang) (Fig. 3). Under loose pieces of coral on seaward tide flat at the north end of Parry Island. March 25, 1960.

TABLE 2

CHROMOSOME NUMBERS PREVIOUSLY REPORTED IN THE OPISTHOBRANCHIATE ORDERS
ENTOMOTAENIATA, CEPHALASPIDEA, ANASPIDEA, SACOGLOSSA, AND SOLEOLIFERA

SPECIES	CHROMOSOME NUMBER		LOCALITY	REFERENCE
	2n	n		
ENTOMOTAENIATA				
Pyramidellidae				
<i>Tiberia fasciata</i>		17	Japan	Inaba, pers. comm.
ANASPIDEA				
Aplysiidae				
<i>Petalifera punctulata</i>	34	17	Japan	Inaba, 1959a
<i>Notarchus leachii freeri</i>		17	Japan	Inaba, 1959a
CEPHALASPIDEA				
Acteonidae				
<i>Cylichnatis angusta</i>		17	Japan	Inaba, pers. comm.
Philinidae				
<i>Philine japonica</i>		17	Japan	Inaba, 1959a
Aglajidae				
<i>Aglaja gigliolii</i>	34	17	Japan	Inaba, 1959a
SACOGLOSSA				
Elysiidae				
<i>Elysia amakusana</i>		17	Japan	Inaba, 1959a
<i>Elysia viridis</i>		17	Italy	Mancino and Sordi, 1964b
Stiligeridae				
<i>Alderia nigra</i>		17	Japan	Inaba, 1961
<i>Hermæopsis variopicta</i>		17	Italy	Mancino and Sordi, 1964b
<i>Placida dendritica</i>	34	17	Italy	Mancino and Sordi, 1964b
<i>Placida viridis</i>	34	17	Italy	Mancino and Sordi, 1964b
<i>Stiliger vesiculosus</i>	34	17	Italy	Mancino and Sordi, 1964b
Juliidae				
<i>Berthelinia limax</i>		17	Japan	Inaba, 1961
Polybranchiidae				
<i>Bosellia mimetica</i>	14	7	Italy	Mancino and Sordi, 1964b
SOLEOLIFERA				
Veronicellidae				
<i>Veronicella floridana</i>		16	U.S.A.	Burch, 1965
<i>Laevicaulis alte</i>		17	India	Natarajan, 1960
Onchidiidae				
<i>Oncidiella kurodai</i>		17	Japan	Inaba, 1961
<i>Onchidium verraculatum</i>	36	18	India	Natarajan, 1959

4. *Stylocheilus longicauda* (Quoy and Gaimard) (Fig. 4). In tide flats of Eniwetok Island. March 4, 1960.

CEPHALASPIDEA

5. *Haminoea linda* Marcus and Burch (Fig. 5). Parry Island, in sand, in about 2 m of water, in lagoon, about 17 m from shore. March 31, 1960.

6. *Haminoea musetta* Marcus and Burch (Fig. 6). Middle part of Parry Island on seaward tide flats. April 2, 1960.

7. *Lathophthalmus smaragdinus* (Rüppel and Leuckart) (Fig. 7). Collected at the south end of Parry Island, under loose pieces of coral on seaward tide flat. March 15, 1960.

8. *Smaragdina calyculata* (Broderip and

TABLE 3
CHROMOSOME NUMBERS OF OPISTHOBRANCHS OBSERVED IN THIS STUDY

SPECIES	CHROMOSOME NUMBER (n)	NUMBER OF SPECIMENS GIVING RESULTS
NUDIBRANCHIA		
Dendrodorididae		
<i>Dendrodoris nigra</i>	13	5
Favorinidae		
<i>Herviella mietta</i>	13 (2n = 26)	1
ANASPIDEA		
Aplysiidae		
<i>Dolabrifera dolabrifera</i>	17	9
<i>Stylocbeilus longicauda</i>	17	2
CEPHALASPIDEA		
Atyidae		
<i>Haminoea linda</i>	17	1
<i>Haminoea musetta</i>	17	2
Smaragdinellidae		
<i>Latobophthalmus smaragdinus</i>	18	2
<i>Smaragdinella calyculata</i>	18	2
SOLEOLIFERA		
Onchidiidae		
<i>Onchidella evelinae</i>	18	2

Sowerby) (Fig. 8). In lagoon at north end of Eniwetok Island.

SOLEOLIFERA

9. *Onchidella evelinae* Marcus and Burch (Fig. 9). In cracks in coral slabs above water line (at low tide) on the lagoon side at the north end of Eniwetok Island. April 5, 1960.

The materials examined consisted of ootestes fixed in either Newcomer's (1953) or Carnoy's (1887) fluids, or the fixative of Sanfelice (1918). The material fixed in Newcomer's or Carnoy's fluids was stained by the acetic-orcein squash technique (La Cour, 1941), and reproductive tissues fixed in Sanfelice's fluid were sectioned at either 8 or 10 micra and stained with Newton's (1926) crystal violet. All observations were made on meiotic cells of spermatogenesis (except in *Herviella mietta*, where spermatogonial cells were also studied) with a Nikon (Nippon Kogaku) microscope equipped with a 100 \times (n.a. 1.25) oil immersion objective and 10 \times , 20 \times , and 30 \times oculars. Drawings were made with the aid of a

camera lucida and reproduced at a table-top magnification of 4260 \times .

OBSERVATIONS

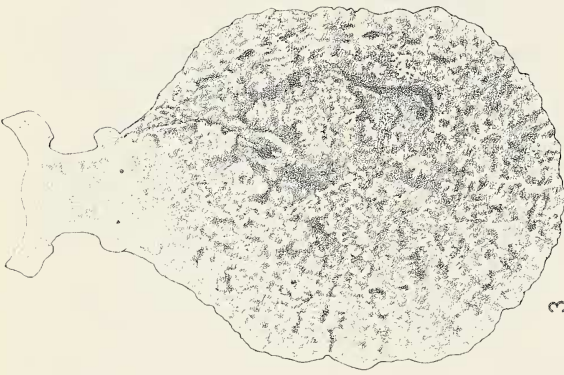
1. *Dendrodoris nigra* (Fig. 10). The five individuals of this species on which we were able to obtain satisfactory observations all had 13 bivalents during prophase of the first meiotic division.

2. *Herviella mietta* (Fig. 11). Only one specimen of this species gave satisfactory results. It had 13 bivalents during diakinesis and 26 chromosomes during metaphase in spermatogonial cells.

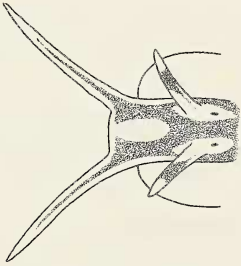
3. *Dolabrifera dolabrifera* (Figs. 12 and 13). Nine specimens had meiotic cells that were satisfactory for chromosome number determinations. All had 17 bivalents during Prophase I and Metaphase I. The chromosomes of a cell during diakinesis from an acetic-orcein squash preparation are shown in Figure 12. Metaphase I bivalents from a cell of paraffin sectioned material are shown in Figure 13.



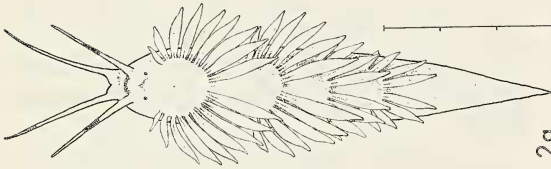
4



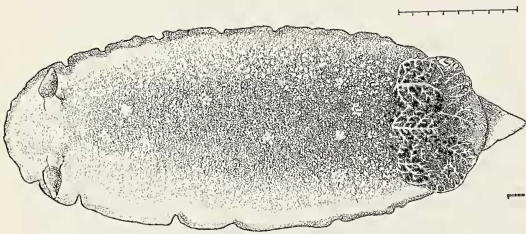
3



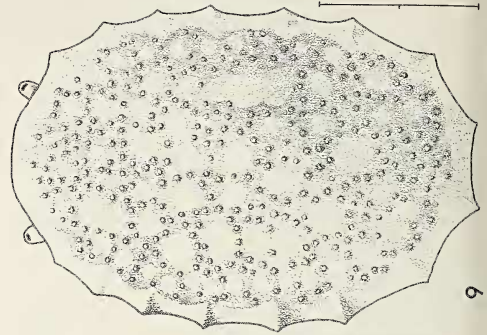
2b



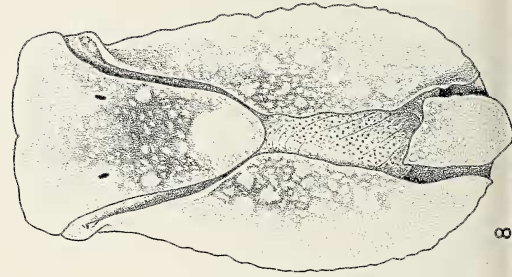
2a



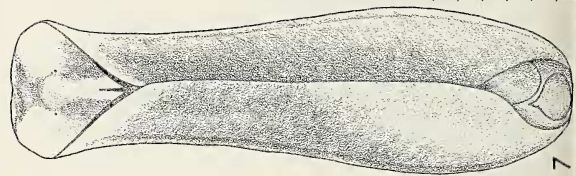
1



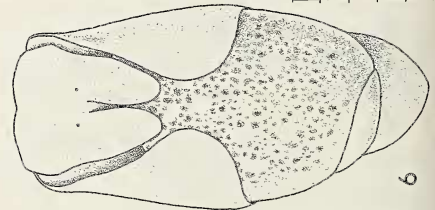
9



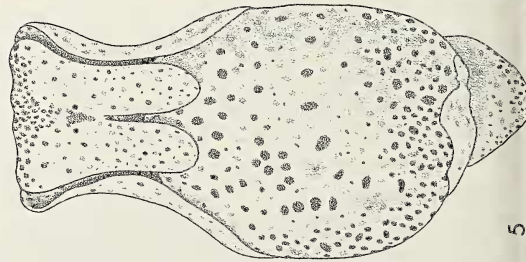
8



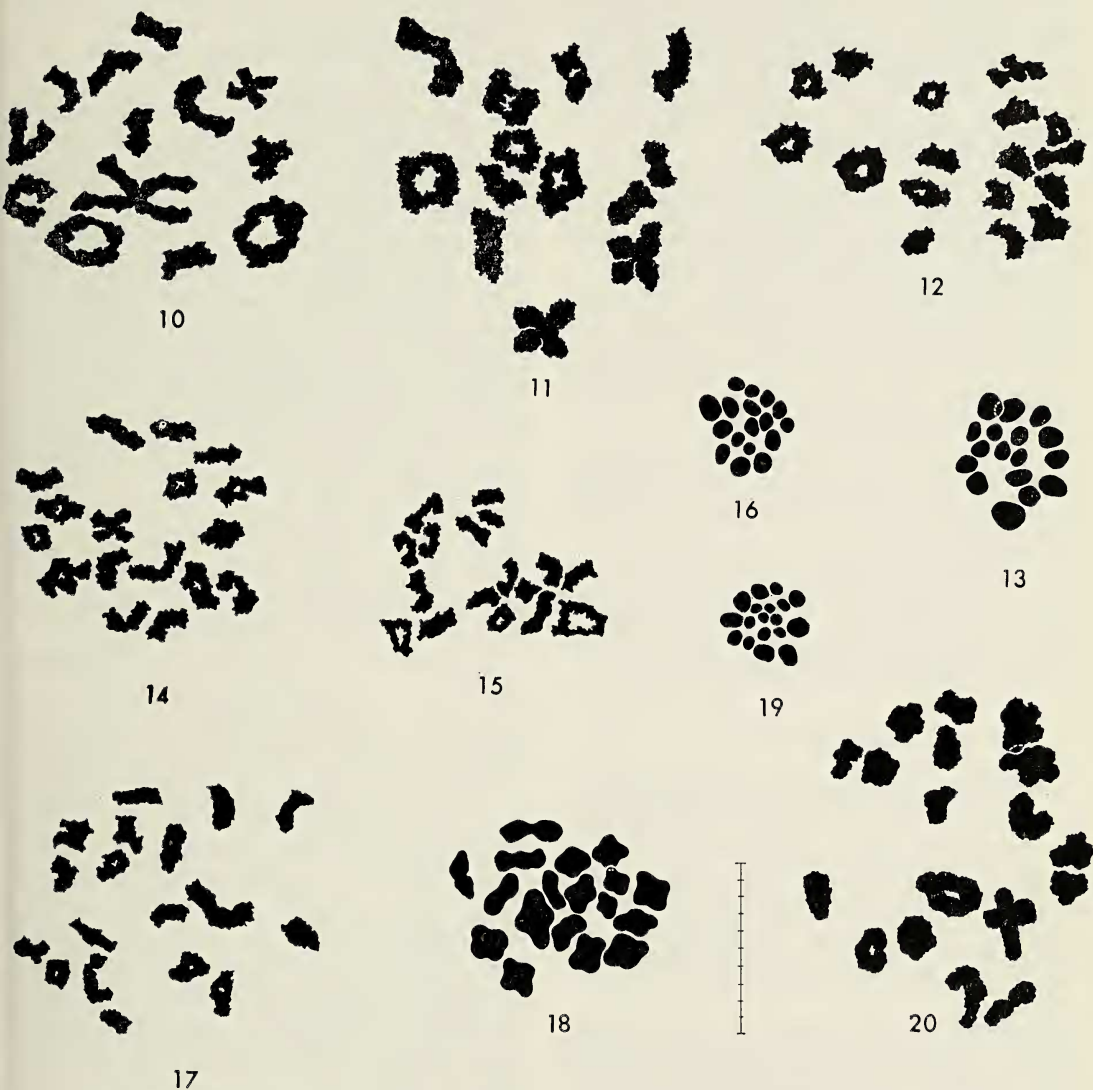
7



6



5



FIGS. 10-20. Chromosomes of Eniwetok opisthobranchs. 10, *Dendrodoris nigra*. 11, *Herviella mietta*. 12 and 13, *Dolabrifera dolabrifera*. 14, *Stylocheilus longicauda*. 15, *Haminoea musetta*. 16, *H. linda*. 17, *Lathophthalmus smaragdinus*. 18, *Smaragdinella calyculata*. 19 and 20, *Onchidella evelinae*.

Figs. 10-12, 14, 15, 17, and 20 are of male diakinesis bivalents; 13, 16, 18, and 19 are of male Metaphase I bivalents. Figs. 10-12, 14, 15, 17, 18, and 20 are from acetic-orcein squash preparations; 13, 16, and 19 are from sectioned material stained with crystal violet. Measurement line divided into micra.

FIGS. 1-9. Eniwetok opisthobranchs used in this study. 1, *Dendrodoris nigra*. 2, *Herviella mietta*. 3, *Dolabrifera dolabrifera*. 4, *Stylocheilus longicauda*. 5, *Haminoea linda*. 6, *H. musetta*. 7, *Lathophthalmus smaragdinus*. 8, *Smaragdinella calyculata*. 9, *Onchidella evelinae*. Measurement lines are divided into millimeters.

4. *Stylocheilus longicauda* (Fig. 14). Satisfactory results were obtained from two specimens. Both had 17 bivalents during diakinesis.

5. *Haminoea linda* (Fig. 16). We were able to obtain cells that were satisfactory for study from only one specimen. These cells had 17 bivalents during Metaphase I.

6. *Haminoea musetta* (Fig. 15). The two specimens studied both had 17 bivalents in cells at the diakinesis stage. The diakinesis bivalents of one such cell are shown in Figure 15.

7. *Latbophthalmus smaragdinus* (Fig. 17). Two specimens had meiotic cells that were satisfactory for chromosome number determinations. All dividing cells from which accurate counts could be made had 18 bivalents.

8. *Smaragdinella calyculata* (Fig. 18). Satisfactory results were obtained from two specimens. Both had 18 bivalents during diakinesis and Metaphase I.

9. *Onchidella evelinae* (Figs. 19 and 20). The two individuals on which we were able to obtain satisfactory observations both had 18 bivalents during diakinesis and Metaphase I.

DISCUSSION

The chromosome numbers of the eight genera of opisthobranchiate mollusks presented here add to the information previously obtained by reliable authors. Of the species studied five belong to three families not studied in past reports, i.e., the Favorinidae (Nudibranchia), and the Atyidae and Smaragdinellidae (Cephalaspidea).

Dendrodoris nigra (Nudibranchia, Dendrodorididae) was studied previously by Inaba and Hirota (1958). We found the same number of chromosomes ($n=13$) for this species from Eniwetok as they reported it to have from Japan. *Herviella mietta* (Nudibranchia, Favorinidae) from Eniwetok also had a haploid number of 13, which adds to the growing information regarding the extreme conservativeness of chromosome numbers of most opisthobranchs. All 16 species of nudibranchs (belonging to nine different families) studied so far have this same haploid number, $n=13$.

Among the Cephalaspidea three species have been studied previously (Inaba, 1959a and personal communication), each belonging to a dif-

ferent family. Each of these three species had a haploid number of 17. In the present investigation two species from each of two additional families were studied. *Haminoea linda* and *H. musetta* (Bullacea, Atyidae) had a haploid chromosome number of 17, but *Smaragdinella calyculata* and *Latbophthalmus smaragdinus* (Philinacea, Smaragdinellidae) each had the haploid number 18. It will be interesting to see if species of the other families of the Philinacea (the Philinidae, Scaphandridae, Aglajidae, Gastropteridae, and Runcinidae) also have 18 pairs of chromosomes. If so, this would separate this superfamily from all other cephalaspideids and, additionally, from all other cytologically known entomotaenids and anaspideids and from most of the sacoglossans. The haploid number 18 in this group may have another significance in that it seems to strengthen Pelseener's (1893) and Boettger's (1955) views regarding the origin of the Basommatophora from the Cephalaspidea, since the haploid number 18 is basic for the Basommatophora.

Dolabrifera dolabrifera and *Stylocheilus longicauda* (Anaspidea, Aplysiidae) both had the haploid number 17, as did the two species studied from this family by Inaba (1959a).

Onchidella evelinae (Soleolifera, Onchidiidae) had a haploid number of 18, which is one bivalent more than that reported by Inaba (1961a) for *O. kurodai* of the same genus from Japan, but $n=18$ is the same number reported by Natarajan (1959) for *Onchidium verraculatum* from India. Much more cytological information is desirable for the various species belonging to this aberrant order, which is sometimes placed with the "pulmonates" (e.g., Baker, 1955).

The conservativeness of chromosome number in the opisthobranchs indicates that these mollusks are extremely resistant to changes in chromosome numbers, regardless of major evolved morphological diversities within the various groups, and that certain major divisions (i.e., the Nudibranchia and the orders with $n=17$ and higher chromosome numbers) have probably been separated for an extremely long geological time. In this regard, *Bosellia mimetica* is either an extremely aberrant species, or its cytological evolution has been much more rapid than has been the evolution of its gross

morphology in respect to all other opisthobranchs so far studied.

ACKNOWLEDGMENTS

Grateful acknowledgment is made to the United States Atomic Energy Commission for supporting the study by the senior author at Eniwetok by providing travel funds, logistical support, and use of the facilities of the Eniwetok Marine Biological Laboratory. The cooperation of the U.S.A.E.C. Eniwetok Field Office and of Holmes and Narver, Inc. greatly facilitated the field collecting. A note of gratitude is due Dr. I. Eugene Wallen, formerly with the U.S.A.E.C., Dr. Robert W. Hiatt, University of Hawaii, and Prof. Henry van der Schalie, University of Michigan, for promoting these studies; to Dr. William H. Heard, Florida State University, for assistance while at Eniwetok; and to John L. Tottenham, Staff Artist, Museum of Zoology, University of Michigan, for preparing illustrations of the animals used in this study.

REFERENCES

- BAKER, H. B. 1955. Heterurethrous and aulacopod. *Nautilus* 68:109-112.
- BOETTGER, C. R. 1955. Die Systematik der euthyneuren Schnecken. *Verh. dtsch. zool. Ges.*, 1954, pp. 253-280.
- BURCH, J. B. 1965. Chromosome numbers and systematics in euthyneuran snails. *Proc. First European Malacol. Congr.*, pp. 215-241.
- CARNOY, H. B. 1887. Conférence donnée à la Société belge de Microscopie. Appendice: I. Les Globules polaires de l'*Ascaris clavata*. *La Cellule, Rec. Cytol. Histol. Gén.* 3(2/3): 227-273.
- INABA, A. 1959a. Cytological studies in molluscs. III. A chromosome survey in the opisthobranchiate Gastropoda. *Annot. Zool. Japan* 32(2):81-88.
- 1959b. On the chromosomes of a nudibranch gastropod, *Dendrodoris* (*Dendrodoris*) *nigra* (Stimpson). *J. Sci. Hiroshima Univ.* 18(9):95-98.
- 1961. Chromosomes of some opisthobranchs. [In Japanese.] *Dobutsugaku Zasshi* 70:24.
- and R. HIROTA. 1954. On the chromosomes of *Glossodoris pallescens*. [In Japanese.] *Dobutsugaku Zasshi* 63:437.
- ——— 1958. A chromosome survey in ten species of nudibranchs (Gastropoda, Mollusca). *Japan. J. Zool.* 12(2):157-162.
- LA COUR, L. 1941. Acetic-orcein: A new stain-fixative for chromosomes. *Stain Technol.* 16: 169-174.
- MAKINO, S. 1956. *An Atlas of the Chromosome Numbers in Animals*. 2nd ed. Iowa State College Press, pp. 22-23.
- MANCINO, G. and M. SORDI. 1964a. Ricerche carilogiche in *Doris verrucosa* Cuvier, 1804 (Gasteropodi, Opisthobranchi) del litorale livornese. *Atti Soc. Toscana Sci. Nat., Ser. B*, 71. 14 pp.
- ——— 1964b. Il corredo cromosomico di alcuni Opisthobranchi Sacoglossi del mar Tirreno. *Ibid.* 71. 12 pp.
- NATARAJAN, R. 1959. Chromosomes of the slug *Onchidium verruculatum* Cuv. (Mollusca: Gastropoda: Pulmonata). *J. Zool. Soc. India* 11(1):30-33.
- 1960. Further cytological studies in Pulmonata (Mollusca: Gastropoda). *Ibid.* 12(1):69-79.
- NEWCOMER, E. H. 1953. A new cytological and histological fixing fluid. *Science* 118(3058): 161.
- NEWTON, W. C. F. 1926. Chromosome studies in *Tulipa* and some related genera. *J. Linn. Soc.* 47(316):339-354.
- PELSENEER, P. 1893. Recherches sur divers Opisthobranches. *Academie royale de Belgique*. Pp. 1-157, pls. 1-25.
- SANFELICE, F. 1918. Recherches sur le Genése des corpuscules du *Molluscum contagiosum*. *Ann. l'Inst. Pasteur* 32(8):363-371.